UNIVERSITY OF PENNSYLVANIA

BACTERIOLOGIC STUDIES IN ENDOCARDITIS

WILLIAM A. KREIDLER

A THESIS

IN BACTERIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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BACTERIOLOGIC STUDIES IN ENDOCARDITIS

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It was the purpose of this study to search for distinctive features in the group of streptococci associated with streptococcus endocarditis. The criteria adopted were morphological, cultural and immunologic characteristics; animal experimentation and toxin formation. Incidentally, the presence of antibodies in the blood of patients suffering from the disease was investigated.

The recognition of a distinct form of endocarditis, has been of comparatively recent date and of slow evolution. Although of only moderately common occurrence, the disease is extremely important, since most of the cases studied have been fatal.

Hemsted [1] in 1855 first suggested an infectious origin for the endocarditis associated with uterine infections, and attention was thereafter directed to the finding of bacteria in the vegetations in both ulcerative and verrucous endocarditis. Heiberg [2] described a case of acute endocarditis which had been reported by Winge in 1869 in which micrococci were found in the vegetations and added another case of acute ulcerative endocarditis in which small micrococci were present in the vegetations.

For a short period it was thought that bacteria were present in all forms of endocarditis. Thus, Klebs [3] felt that he could separate rheumatic from septic cases by the character of the cocci found in microscopic sections. Koster [4] thought that verrucous endocarditis was a chronic and healed stage of ulcerative endocarditis and hence originally of bacterial origin. It may be noted that this idea of healed bacteria-free endocarditis has been supported with new evidence in recent years by Libman [5] but with the distinction that the healed cases are supposed to have been of the subacute bacterial type.

Later investigators failed to demonstrate bacteria in the lesions of verrucous endocarditis. Hamburger [6] examined ten such cases with

Received for publication, April 29, 1926.

1 Lancet, 1913, 1, p. 10.
2 Virchows Arch. f. path. Anat., 1872, 56, p. 407.
3 Arch. f. exper. Path. u. Pharmakol., 1878, 9, p. 52.
4 Virchows Arch. f. path. Anat., 1878, 72, p. 257.
5 Am. Jour. Med. Sc., 1913, 146, 525.
6 Ueber akute Endocarditis in ihrer Beziehung zu Bacterien, Berlin, 1879.

negative results and Weichselbaum [7] questioned whether verrucous endocarditis was due to microbic invasion. This view received further support from Wyssokowitch [8] and Orth.[9]

Additional interest was lent to the discussion by the experimental production of acute endocarditis by previously puncturing the aortic leaflets according to a method devised by Rosenbach.[10] Wyssokowitch [8] was able to infect the injured valve by injecting pyogenic streptococci. Ribbert [11] produced acute endocarditis merely by injecting a suspension of staphylococci and fine particles of potato intravenously. Many reports of the production of endocarditis by injecting cultures of streptococci have been subsequently published.

As the facts then stood, there were two general types of endocarditis, namely, those associated with bacteria and those in which no direct connection with bacteria could be proved. The introduction of blood culture methods was responsible for separating the bacterial group into acute and chronic cases. Of the early reports, that of Kühnaw [12] is noteworthy, in that 66 cases of rheumatism, many of which were accompanied by endocarditis, gave negative cultures, the finding of sarcinae, staphylococci and Bacillus subtilis being discounted.

It soon became evident that among those cases of endocarditis which gave definitely positive blood cultures a certain group could be distinguished because of their subacute or chronic character. These cases most frequently yielded streptococci from blood cultures. This was noted by Harbitz.[13] Later Horder [14] very accurately described cases of acute and chronic infectious endocarditis of which 17 were subacute and chronic and yielded blood cultures of "saprophytic streptococci." In the following year Schottmüller [15] individualized this type of endocarditis with "endocarditis lenta," a disease of slow, insidious onset, definite bacteriology and symptoms of injured heart valves with bacteremia. The five cases which he described were caused by streptococci of the green-producing variety. In the same year Libman and Celler [16] described the same disease under the name of "subacute bacterial" endocarditis and found later [17] that while most of the streptococci

[7] Centralbl. f Bakteriol., I, O., 1887, 2, p. 209.
[8] Virchows Arch. f. path. Anat., 1886, 103, p. 301.
[9] Ibid., p. 333.
[10] Arch. f. exper. Path. u. Pharmakol., 1878, 9, p. 1.
[11] Deutsch. med. Wchnschr., 1885, 11, p. 717.
[12] Ztschr. f. Hyg. u. Infektionskrankh., 1897, 25, p. 492.
[13] Deutsch. med. Wchnschr., 1899, 25, p. 121.
[14] Quart. J. Med., 1908-1909, 2, p. 289.
[15] München. med. Wchnschr., 1910, 57, p. 617.
[16] Am. J. M. Sc., 1910, 140, p. 516.
[17] Ibid., 1912, 144, p. 313.

produced green on blood agar plates, some were indifferent and some produced slight clearing. They also stated that the streptococci found were not very virulent.

While the bacteria obtained are almost always streptococci, other organisms, such as influenza bacilli, pneumococci and gram-negative cocci have been described.[18] The cases on which the present study was based were all cases of streptococcus infection. The characteristics of these streptococci have been studied by others, and such features as low virulence and their effect on blood agar have been described. Horder [14] studied the fermentation reactions of the streptococci from seventeen such cases from the standpoint of the grouping proposed by Andrews and Horder [19] and found that nine belonged to the salivarius type and eight to the fecalis type. Major [20] studied the complement-fixing antibodies in the serum of a patient and concluded that the reaction was not specific, since it was also obtained with an antigen made from Streptococcus pyogenes. Rosenow [21] claims that the bacteria usually isolated from these cases are "modified pneumococci," and tries to show that "the continuation of infection and death are largely due to a process of bacterial immunization against the antibodies of the host rather than to the virulence in the usual sense of the infecting bacteria."

The fourteen cases herein described were typical of the disease. A brief synopsis of each case follows:

CASE 1.—Colored, aged 19, single, came into the hospital complaining of shortness of breath, sharp pains over the precordia, and aching over body, particularly in the joints. She had chicken pox, whooping cough, tonsillitis frequently, influenza five years ago, and rheumatic fever at 15 years of age. She also had dropsy at this time and frequently spat blood. Nocturia for the past eight months and polyuria (4-5 times during day). She weighed 145 pounds and had fluctuating fever while in the hospital (3 months). There was a diastolic murmur over aortic valve area and systolic murmur at the apex. The joints were very tender. Streptococcus viridans was isolated from blood culture and vegetations on the mitral valve. The Wassermann test was negative, blood urea 32, uric acid 7, sugar 95. Clinical diagnosis: acute endocarditis, aortic and mitral insufficiency, cardiac hypertrophy and decompensation.

CASE 2.—Colored laborer, entered the hospital complaining of pains in chest, had slight fever, cough and expectoration. Previously he had measles, whooping cough, mumps. He was emaciated, had râles over bases of both lungs, loud systolic murmurs and pericardial friction rub. The heart appeared strong and overacting. The clinical diagnosis was subacute endocarditis and lobar pneumonia. Pneumococcus group 4 was isolated from the sputum and Streptococcus viridans from the blood. The Wassermann test was negative. The urine contained a trace of albumin and granular casts. He left the hospital at the end of a month greatly relieved.

CASE 3.—Negress, 45 years of age. Her chief complaint was shortness of breath and swelling of feet. She had smallpox, chicken pox, measles, mumps and whooping cough, typhoid fever two years previously and malaria during the past year. She had two children and two miscarriages. The present trouble began a year ago when patient had her menopause. She had hot flushes, air hunger, hot feet and palpitation of heart. Five months ago she had an attack of indigestion and vomited a great deal of fluid. Since then she has been nervous and unable to sleep soundly. Her tonsils were very much enlarged. Small amount of fluid was drained from

[18] Simons, I.: Quart. J. Med., 1913-1914, 7, p. 291.
[19] Lancet, 1906, 2, p. 775.
[20] Bull. Johns Hopkins Hosp., 1912, 23, p. 326.
[21] J. Infect. Dis., 1909, 6, p. 245; 1910, 7, p. 429.

the peritoneal cavity and she had a draining sinus. Three days before death she became stuporous and it was impossible to clear up her edema. Streptococcus viridans was isolated from the blood. Wassermann test was negative and blood chemistry was normal. Clinical diagnosis was subacute endocarditis and chronic nephritis.

CASE 4.—Patient, aged 29, complained of pain in joints (knees and ankles). He had a systolic murmur. Wassermann and colloidal gold tests were negative. The urine showed a heavy cloud of albumin and hyalin casts. Blood urea was 40, uric acid 3.8. Unable to get early history. At necropsy vegetations were found on the mitral valve. Streptococcus viridans was isolated from the vegetations and from a splenic infarct. The clinical diagnosis was subacute endocarditis and acute nephritis.

CASE 5.—White laborer, aged 42. For past year patient complained of pains in chest and shortness of breath. He had had common childhood diseases. His tonsils were enlarged and ragged. The Wassermann test was negative; blood chemistry normal; blood culture negative. At necropsy Streptococcus pyogenes was isolated from the vegetations on the mitral valve. This organism later lost its ability to hemolyze blood cells and became indifferent on blood agar. Clinical diagnosis was subacute endocarditis.

CASE 6.—Widower with no children, aged 58. Had pains in chest and ankles. Feet were constantly perspiring and temperature usually 99 F. He had typhoid and chancroid 18 years ago and pleurisy recently. Had an enlarged prostate gland and eye reaction to lues but denies chancre. He had a systolic heart murmur. Streptococcus viridans isolated from blood culture. The Wassermann test was negative; urine showed faint trace of albumin; sputum negative for tubercle bacilli. Diagnosis was subacute endocarditis.

CASE 7.—Patient, aged 81 years, was in the hospital only one week before she died. History is incomplete. She was emaciated and mentally abnormal. Blood culture at autopsy showed Streptococcus viridans. Clinical diagnosis was subacute endocarditis, general arteriosclerosis and senility.

CASE 8.—This strain was received from another hospital and history is incomplete. The clinical diagnosis was subacute endocarditis and pregnancy. Streptococcus viridans was isolated from the blood. Systolic murmur was quite marked.

CASE 9.—Colored laborer, aged 27, came into the hospital complaining of a swollen and painful ankle and shortness of breath (3-4 months). Had measles and malaria at 17 years of age. Has been married two years and has no children. Had gonorrhea four years ago, tonsillitis two years ago. Nocturia (4-5 times). He had a loud systolic murmur over apex of heart. Had moderate sclerosis. Denied lues. Temperature was irregular, almost septic. His Wassermann was four plus; had leukocytosis; large amount of albumin in urine; blood chemistry normal. Streptococcus viridans was isolated from blood culture twice and from vegetations on the mitral valve at necropsy. Clinical diagnosis was acute endocarditis and syphilis.

CASE 10.—Patient, 24 years of age. Had endocarditis ten years previously with long convalescence and recovery. As a member of the merchant marine, he led the roughest kind of life, and during this time contracted both syphilis and gonorrhea. He was admitted to the hospital with a septic temperature but apaprently not acutely ill. Two blood cultures showed Streptococcus viridans. He was discharged at the end of three months and is now apparently leading a normal life. Clinical diagnosis was subacute endocarditis and syphilis.

CASE 11.—This was an ambulatory case of chronic endocarditis. Streptococcus viridans was isolated from the blood.

CASE 12.—Colored patient, aged 23, came into the hospital talking at random and had pains in the head. Had chancre and gonorrhea nine years ago. Moist râles were heard in apex of right lung. The heart had a distinct diastolic murmur. Ran a continued elevated temperature. Corrigan pulse was pronounced. Wassermann test was negative; urine showed heavy trace of albumin and a few hyaline casts. Streptococcus viridans was isolated from the blood on two occasions and from the mitral valve vegetations at necropsy. Clinical diagnosis was subacute endocarditis and chronic nephritis.

CASE 13.—A 15 year old girl who died after three weeks in the hospital. She had acute rheumatic fever when 12 years old, was emaciated and teeth were in bad condition. Heart was very large and Streptococcus viridans was isolated from the blood. She had nocturia (4.5 times). Acute sore throat troubled her frequently. Clinical diagnosis was acute endocarditis and anemia.

CASE 14.—Colored man, 30 years of age, fairly well developed, had infected pharynx and fetid breath. Physical examination showed moist râles over upper lobe, apex of heart an inch outside of nipple line, systolic murmur. He complained of shortness of breath, a cold with pain in right chest and coughed up yellowish mucoid sputum. He never had an infectious disease. While in the hospital he developed acute arthritis, his left elbow becoming swollen, hot and tender. He had fever constantly. Urine analysis showed trace of albumin and few casts. Wassermann test was negative; sputum negative for tubercle bacilli. Streptococcus viridans was isolated from two blood cultures during life, and from the vegetations on the mitral valve and heart blood at autopsy. Clinical diagnosis was subacute endocarditis and lobar pneumonia.

CULTURE MEDIUMS

Beef Infusion Broth.—500 grams of finely chopped lean beef was placed in container with 1000 cc. of water and allowed to stand in the ice box overnight. The infusion was then boiled thirty minutes over an open flame, filtered through gauze and then through wet filter paper to remove the fat. Volume was brought up to 1000 cc. by the addition of water. To this infusion was added 10 gm. peptone (Difco), 5 gm. sodium chloride. Mixture was boiled over open flame until ingredients were dissolved and reaction adjusted to P_H 7.8., then filtered through filter paper and given fractional sterilization in the Arnold.

Brain Broth.—To 1000 cc. infusion broth was added 10 gm (1%) glucose. Long test tubes were three-quarters filled and several pieces of brain added. (Calf brain was cut into blocks about one cc. in size and washed in running water to remove blood.) These tubes were sterilized in the autoclave at twelve pounds for twenty minutes.

Trypsinized Broth.—To 1000 cc. sterile infusion broth was added 10 cc. of a 10% solution of trypsin in normal sodium chloride which had previously been sterilized by passing through a porcelain filter (Mandler 4).

CULTURE METHODS

Blood Culture.

Twenty cc. of blood was the usual amount taken for cultural purposes. Of this quantity:

3 cc. was placed in 75 cc. infusion broth with reaction of P_H 7.6.

1 cc. in tube of brain broth (P_H 7.6).

1 cc. in tube of trypsinized broth (P_H 7.6).

10 cc. was allowed to clot and the latter dropped in 50 cc. dextrose (0.2 %) beef infusion broth (P_H 7.6). This is a modification of Clawson's[22] method. The clot seems to provide partial anerobic conditions and any antibodies present are diluted in fluid medium. The serum separated was used for agglutination tests.

Concentration Method—To 5 cc. of blood was added 45 cc. sterile distilled water. This tube was then centrifugalized at high speed for ½ hour. After discarding the supernatant liquid, the residue was emulsified in 10 cc. infusion agar (P_H 7.6) melted and cooled to 45 C. and a plate poured.

These cultures were incubated at 37.5 C. The streptococci usually grew out in less than five days but no cultures were considered negative until they remained sterile at the end of one month's incubation.

Cultures at the Autopsy Table.

Heart Blood Cultures—Blood was collected with the utmost precautions direct from the heart chamber after searing the outside of the heart and and an opening through its wall. The blood was collected in a sterile pipette and placed in 75 cc. glucose broth. Subcultures were made from this broth on blood agar plates.

Infarcts of Spleen, etc.—The surface of the spleen over the infarct was seared and an opening made through the capsule with a hot knife. A stiff inoculating wire was inserted well beyond the depth of the stab puncture made by the hot knife and blood agar plates inoculated with the material collected.

[22] J. Infect. Dis., 1925, 36, p. 446.

Vegetations of the Heart Valve. —Forceps and scissors were sterilized in the flame and portions of the vegetations cut off and placed in a sterile petri dish. From this material blood agar plates and broth tubes were inoculated. Cultures were incubated at 37.5 C.

MORPHOLOGIC AND CULTURAL CHARACTERISTICS

Most workers who have isolated organisms from endocarditis patients report diplococci or streptococci. Poynton and Paine [23] called their organism a diplococcus. The action on the blood agar plate is not well described in the older literature. Beaton and Walker [24] and Walker and Ryffel [25] have described their strains as producing a brownish discoloration. Lyall [26] found his strains to be inactive on blood. Rosenow [27] reported strains some of which were inactive on blood,

TABLE 1

ORIGIN OF STRAINS OF STREPTOCOCCUS VIRIDANS

	Source of Culture	Clinical Diagnosis	Pathological Diagnosis
1	Blood.....	Acute endocarditis.....	Acute endocarditis; aortic and mitral insufficiency; cardiac hypertrophy and decompensation
2	Blood.....	Subacute endocarditis; lobar pneumonia	Subacute endocarditis; lobar pneumonia
3	Blood (at necropsy)...	Subacute endocarditis; chronic nephritis	Subacute endocarditis; chronic nephritis
4	Splenic infarct.....	Acute endocarditis; acute nephritis	Acute endocarditis with septic infarcts in spleen, kidney and myocardium
5	Vegetations on mitral valve	Subacute endocarditis...	Subacute endocarditis
6	Blood.....	Subacute endocarditis	
7	Blood (at necropsy)...	Subacute endocarditis...	Subacute endocarditis; cardiac decompensation
8	Blood.....	Subacute endocarditis; pregnancy	
9	Blood and vegetations on mitral valve	Acute endocarditis; syphilis	Acute endocarditis; syphilis
10	Blood.....	Subacute endocarditis	
11	Blood.....	Chronic endocarditis (ambulatory case)	
12	Blood and vegetations on mitral valve	Subacute endocarditis; chronic nephritis	Subacute endocarditis; chronic nephritis
13	Blood.....	Acute endocarditis; anemia	
14	Blood.....	Subacute endocarditis; lobar pneumonia	

some produced green, while others were hemolytic. Swift and Kinsella [28] found that their organisms belonged in the viridans group. They believe that the streptococci described by earlier workers correspond to what is now known as Streptococcus viridans.

The biologic tests, as reported in the literature, have shown that not all organisms isolated from endocarditis patients belong to the same group. Andrewes and Horder [29] found that one of their strains belonged to the buccalis group and one to the fecalis group. Beattie and Yates [20] reported three strains as belonging

[23] Lancet, 1910, 1, p. 1524.
[24] Brit. M. J., 1903, 1, p. 237.
[25] Brit. M. J., 1903, 2, p. 659.
[26] J. M. Res., 1914, 30, p. 487.
[27] J. Infect. Dis., 1914, 14, p. 61.
[28] Arch. Int. Med., 1917, 19, p. 381.
[29] J. Path. & Bacteriol., 1911-1912, 16, p. 247.

to the fecalis group. Lyall [28] reported the fermentation reactions of five organisms isolated from endocarditis. Three of the five fermented mannite and were grouped as fecalis. Quigley [30] examined 6 strains, 4 of which fermented mannite. The organisms reported in the literature are usually streptococci, nonhemolytic in character.

Most of my strains when first isolated were arranged as diplococci. They have a tendency to be lancet shaped but differ from the pneumococcus in not being bile soluble, in not fermenting inulin (with one exception) and in not being capsule producers. Although these strains at times grew out in short chains there is a marked tendency for them to appear as diplococci. In only one strain (5) which displayed hemolytic properties at first, was there any tendency towards spontaneous agglutination in broth cultures.

TABLE 2

CULTURAL CHARACTERISTICS OF STRAINS OF STREPTOCOCCUS VIRIDANS

Bile insoluble organisms did not liquefy gelatin, and were not fatal to mice in 2 cc. amounts.

	Plain Broth	Blood Agar	Litmus Milk
1	Turbid diplococci	Green	Acid
2	Turbid short chains	Green	Acid coagulated
3	Turbid short chains	Green	Acid coagulated
4	Turbid short chains	Green	Acid coagulated
5	Clumped at bottom	Hemolytic; indifferent	Acid
6	Turbid diplococci	Green	Acid coagulated
7	Turbid diplococci	Green	Acid coagulated
8	Turbid short chains	Green	Acid coagulated
9	Turbid short chains	Green; indifferent	Acid coagulated
10	Turbid diplococci	Green	Acid
11	Turbid short chains	Green	Acid coagulated
12	Turbid short chains	Green	Acid coagulated
13	Turbid diplococci	Green	Acid coagulated
14	Turbid diplococci	Green; indifferent	Acid

With one exception all my strains produced a green discoloration on the blood agar plate at 24 hours incubation when first isolated. They correspond to the alpha type of Brown.[31] One strain (5) was a typical beta hemolyzer. This strain was found in pure culture in the vegetation on the mitral valve of a patient suffering from subacute endocarditis. Jackson [32] reported the production of typical endocarditis lesions in the myocardium of rabbits by the injection of hemolytic streptococci.

None of the strains liquefied gelatin and all produced acid in litmus milk. The fermentation reactions are recorded in table 3. The mediums used were infusion broth and Hiss serum water with 1% of the carbohydrates listed. Brom-thymol-blue was added to serve as indicator. Readings were taken after two, six and ten days. According to

[30] J. Infect. Dis., 1918, 22, p. 198.
[31] Monograph No. 9, Rockefeller Inst. for M. Research, 1919.
[32] J. Infect. Dis., 1912, 11, p. 243.

Holman's [33] classification three strains were fecalis, four were mitis, one was salivarius, three were nonhemolyticus 1, two were nonhemolyticus 11, one was nonhemolyticus 111. When considering all the sugars used by Brown, however, no classification into groups appears possible on the basis of carbohydrate fermentation.

CULTIVATION OF STRAINS IN PERITONEAL CAVITY OF RABBIT

The strains which figured in the experiments herein described were collected over a period of eighteen months. Consequently it was thought advisable to determine whether prolonged cultivation upon blood agar had any effect on the cultural characteristics and serological reactions of the strains. Two strains were picked at random and placed in collodion sacs in the peritoneal cavity of rabbits. At the end of six

TABLE 3

FERMENTATION REACTIONS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Glucose	4	2	4	4	4	4	4	4	4	4	4	4	4	4
Lactose	4	2	4	4	4	4	4	4	0	0	4	4	4	0
Sucrose	4	4	4	4	4	4	4	4	4	2	4	4	4	2
Mannite	4	2	2	0	0	0	4	4	4	4	4	0	0	4
Salicin	4	0	0	4	2	4	4	0	4	0	4	0	4	4
Inulin	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Raffinose	4	0	0	4	0	4	0	0	0	4	4	2	4	0

Maximum fermentation 4; small amount fermentation 2; negative 0. All controls were negative.

months they were removed and their characteristics compared with those of the same strains when first isolated and when preserved on blood agar.

The collodion sacs for this purpose were made by heating a piece of glass tubing three centimeters in length and joining it to one end of a one-half ounce horse capsule. After cooling, the capsule was dipped in collodion until a sufficiently durable sac was obtained (five dippings). Then the covered capsule was placed in water bath (50-60 C.) and the liquefied gelatin removed from the sac by means of a capillary pipette. The open end of the glass tube was then plugged with cotton and the sac half filled with water and sterilized in the autoclave. The water was removed and replaced with a 24 hour broth culture of 4 and another with 6. The glass tubes were then sealed off in the flame and the sacs inserted into the peritoneal cavity of a rabbit.

[33] J. Med. Res., 1916, 34, p. 377.

The collodion sac permits the products of bacterial metabolism to diffuse outward and the body fluids to diffuse inward through its walls. At the same time leucocytes cannot pass through the sac to attack the bacteria, thus affording favorable conditions for growth.

After six months in the peritoneal cavity the strains were recovered and found to resemble the homologous strains preserved on blood agar, in cultural characteristics as well as yielding the same serological reactions. It is therefore apparent that whether grown on artificial media or in an animal these characteristics of a particular strain remain unchanged.

SEROLOGIC REACTIONS

Preparation of Immune Serums.—Eleven of the 14 strains isolated from patients suffering from endocarditis were used to immunize rabbits. For this purpose the bacteria were grown in infusion broth (P_H 7.6), and the sediment after centrifugation resuspended in 0.85% NaCl. These suspensions were standardized to a turbidity of one billion per cc., heated to 53 C. for one hour and then preserved in the ice-box.

The rabbits were injected first subcutaneously, then intravenously. On consecutive days 0.25, 0.5, and 1.0 cc. of each suspension was injected subcutaneously into a series of animals. After a rest period of four days intravenous injections were begun and amounts of 0.25, 0.5, and 0.5 cc. were injected. Following another rest period of four days 0.5, 0.75 and 1.0 cc. were injected intravenously, et cetera.

The blood serum of each rabbit was tested from time to time for agglutinin titer and at the end of five weeks the titer was deemed sufficiently high to yield satisfactory results. The serums were obtained by bleeding the rabbits from the heart and preserved by adding an equal volume of 0.6% phenol.

Agglutination.—In determining the titer of each serum the same antigens were used that had served for immunization. To a series of tubes containing dilutions of immune serum ranging from 1:20 to 1:10,240 was added 0.5 cc. of homologous antigen. The dilutions of serum were made in 0.85% NaCl. The tests were made in the water bath at 50 C. with the depth of the water so adjusted that the level of the liquid in the agglutination tubes stood out somewhat above the surface of the water in the bath, thus bringing into play the slight convection currents within the tubes which appear so effective in the

development of sharp end results in the agglutination reaction. Readings were made after six hours and confirmed after 18 hours in the bath.

Tests were conducted with antigens of living bacteria with practically no difference in results. Controls of normal rabbit serum were used with each antigen. No proagglutinoid zone was observed in any case. The titer of each serum is shown in table 4.

Cross agglutination was tried in the attempt at grouping of the strains. The 11 agglutinating serums which had been prepared were used with 14 strains. Results are found in table 5. The test was performed at dilutions 1:10, 1:160, 1:320, 1:500 with uniform results. Each strain was agglutinated only by its homologous serum indicating a high degree of specificity among organisms isolated from patients suffering from endocarditis and possessing similar morphologic and cultural characteristics. This finding is in agreement with Krum-

TABLE 4

RESULTS OF AGGLUTINATION WITH HOMOLOGOUS SERUMS

Dilutions of Serum	1	2	3	4	5	6	7	8	9	11	12
1:160	4	4	4	4	4	4	4	4	4	4	4
1:320	3	4	4	4	4	4	4	4	4	4	4
1:640	2	4	4	4	3	3	4	4	4	4	4
1:1280	0	4	2	4	0	2	2	4	4	4	2
1:2560	0	3	2	0	0	0	0	3	3	4	2
1:5120	0	0	0	0	0	0	0	0	2	0	0
1:10240	0	0	0	0	0	0	0	0	0	0	0

Complete agglutination 4; partial agglutination 3, 2; no agglutination 0. All controls were negative.

wiede and Valentine,[34] Kinsella,[35] Norton,[36] Clawson,[22] Beattie and Yates,[37] Swift and Kinsella,[28] and others. On the basis of agglutination it is quite evident that the strains of Streptococcus viridans discussed in this paper form a heterologous group.

Absorption of Agglutinins.—Although no cross-agglutination was obtained with the strains investigated, it was deemed advisable to determine whether any absorption of agglutinins might take place. Norton[36] in his work on the relationship of the members of the viridans group found the absorption method more reliable than simple agglutination.

Serums 1, 2, and 4 were tested with negative results. Each of the above mentioned serums was diluted 1:50 with a 24 hour broth culture of each of the strains 1 to 14. As a control, the serum was diluted

[34] J. Infect. Dis., 1916, 19, p. 217.
[35] Arch. Int. Med., 1917, 19, p. 367.
[36] J. Infect. Dis., 1923, 32, p. 37.
[37] J. Path. & Bacteriol., 1912-1913, 17, p. 538.

1 : 50 with uninoculated broth. The absorption reaction period was four hours in the incubator at 37.5 C. with repeated shaking, and 18 hours in the ice-box. The suspensions were then centrifuged and the sedimented organisms discarded. Each serum which had been exposed to the other strains for absorption was then tested for effects on its titer for its own antigen. No absorption of the agglutinins of the three serums tested had taken place so it was not deemed necessary to continue with the remaining serums.

Precipitin Reactions.—The method of preparation of antigens for the precipitin reaction was a modification of that of Vaughan.[38] Flat bottles containing blood infusion agar were inoculated with 1 cc. of a 24 hour broth culture of strains 1 to 7. After being incubated 48

TABLE 5

RESULTS OF CROSS AGGLUTINATIONS

Serums	Antigens													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	4	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	4	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	4	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	4	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	4	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	4	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	4	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	4	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	4	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	4	0	0

Complete agglutination 4; no agglutination 0. All controls were negative.

hours at 37.5 C. the growth on the surface of the agar was washed off with 0.85% NaCl and centrifugalized. The organisms were then resuspended successively in 50% alcohol, 70% alcohol, 95% alcohol. The organisms were then placed in a Soxhlet extractor and the fat abstracted with absolute alcohol (one day), then with ether (three days). The residue was ground in an agate mortar and the powder kept in the ice-box. As needed the cellular substance was disrupted in 0.5% KOH (15 times the weight of the powder). The alkali was neutralized with dilute HCl. Precipitation did not occur in as high dilutions as did agglutination with the homologous serums and no cross-precipitation whatever took place.

Complement Fixation Reactions.—The antigens used in these experiments were the same as those used in the immunization of rabbits and

[38] Protein Split Products, 1913.

in the agglutination reactions. All antigens were first tested for hemolytic activity by mixing varying quantities of each antigen with sheep cells and sufficient NaCl solution to bring contents of liquid in all tubes up to a uniform volume. There was no hemolysis in any case. The immune serums were tested for any natural amboceptor against sheep cells by performing the usual fixation test with serum, complement, sheep cells and NaCl solution, but omitting the addition of amboceptor. There was no hemolytic amboceptor present in any of the immune serums.

After going through the preliminaries of obtaining antigenic and anti-complementary doses, the tests were made by incubating at 37 C. It was found that all immune serums gave fixation with homologous

TABLE 6

RESULTS OF COMPLEMENT FIXATION TESTS

	Antigens												
Serums	1	2	3	4	5	6	7	8	9	10	11	12	13 & 14
1.....	2	0	0	0	0	0	0	0	0	0	0	0	0
2.....	0	4	0	0	0	0	0	0	0	0	0	0	0
3.....	0	0	4	0	0	0	0	0	0	0	0	0	0
4.....	0	0	0	4	0	0	0	0	0	0	0	0	0
5.....	0	0	0	0	4	0	0	0	0	0	0	0	0
6.....	0	0	0	0	0	4	0	0	0	0	0	0	0
7.....	0	0	0	0	0	0	2	0	0	0	0	0	0
8.....	0	0	0	0	0	0	0	4	0	0	0	0	0
9.....	0	0	0	0	0	0	0	0	4	0	0	0	0
11.....	0	0	0	0	0	0	0	0	0	0	4	0	0
12.....	0	0	0	0	0	0	0	0	0	0	0	4	0

No hemolysis 4; partial hemolysis 2; complete hemolysis 0. All controls were negative.

antigens. No cross-fixation, however, took place, which result supports those of agglutination and precipitation (table 6). From the serologic tests, it is quite evident that the members of the viridans group worked with form a heterologous group.

Immune Bodies in Patients' Serum.—It is surprising how little work has been done to determine the incidence of immune bodies in the serums of endocarditis patients. Tunnicliff [39] found that opsonins for streptococci were increased in the blood of patients having endocarditis. In the serum of seven of twelve patients, she found agglutinins for the homologous organisms. Kinsella [35] found in testing the blood of 12 patients having subacute bacterial endocarditis that all showed the presence of agglutinins. He used no dilutions over 1 : 40. Clawson [22] tested five cases. In four, agglutinins were found in a dilution of 1 : 50 or more. In one, they could not be detected.

[39] J. Infect. Dis., 1909, 6, p. 346.

I have tested four cases. In three, agglutinins were found in all dilutions up to 1:2560 and in the other up to 1:5120. In two cases streptococci of the viridans group were isolated from the blood during life, and from the heart blood and vegetations on the mitral valve at necropsy. For all three strains in each case there were agglutinins found in high dilutions of the patients' serum. These cases were all found to be typical at necropsy.

ANIMAL INOCULATIONS

In considering whether the streptococcus may be the etiologic agent in producing bacterial endocarditis, much importance rests on whether lesions similar to human endocarditis lesions can be produced experimentally.

Many investigators have produced endocarditis in rabbits and a few in dogs and monkeys. Poynton and Payne believe that the same organism may produce either simple (rheumatic) endocarditis or malignant (subacute bacterial) endocarditis. Beattie and Dickson [40] report experimental data to show that an organism isolated in a case of subacute bacterial endocarditis may when injected into one animal produce a simple endocarditis and when injected into another animal, a malignant endocarditis. The work of Cole,[41] Davis,[42] Jackson,[32] and Henrici [43] shows that streptococci from other sources are capable of producing endocarditis.

The microscopic myocardial lesions appear to be the most difficult to reproduce. Thalhimer and Rothschild [44] have produced nodular lesions in the myocardium of rabbits by injecting streptococci, but they do not consider these lesions similar to the typical Aschoff nodule. They think their lesions and the lesions of Bracht and Wachter [45] have neither the structure, location, typical cells nor staining reaction characteristic of the Aschoff nodule. But Coombs, Miller and Kettle,[46] Jackson,[32] and Rosenow [27] report that they have produced the Aschoff nodules in the myocardium of rabbits, and that these nodules are typical in all respects of those found in man in acute bacterial endocarditis.

Clawson [22] and Swift [47] give a good description of the pathological picture in man. The gross rheumatic valvular lesion in man consists of

[40] Special Pathology, 1926, 2, p. 507.
[41] J. Infect. Dis., 1904, 1, p. 714.
[42] Ibid., 1912, 10, p. 148.
[43] J. Exper. Med., 1916, 19, p. 572.
[44] Ibid., 1914, 19, p. 429.
[45] Deutsch. Arch. f. klin. Med., 1909, 96, p. 493.
[46] Lancet, 1912, 2, p. 1209.
[47] Swift: J. Exper. Med., 1924, 39, p. 497.

small smooth globular masses on the free margins and at the points of contact of the valves. The microscopic lesions consists of proliferative and exudative inflammation which begins within the valve proper, often some distance beneath the endothelium. The proliferation may be moderate or pronounced. The exudation consists of fibrin and cells, mostly large mononuclear and multinuclear cells though small lymphocytes and a few polymorphonuclear cells are generally present. There is usually a necrotic center in the nodule of inflammation. This necrosis may be found in a degree which can scarcely be noticed or in so extensive a degree that the center of the nodular mass on a valve may have undergone complete necrosis. The endothelium usually ruptures, and a small thrombotic mass from the blood content may collect. The inflammation in a cardiac valve is essentially that of the Aschoff nodule or of a subcutaneous nodule. In the valve, the nodules of inflammation are multiple. With these conditions in mind, the lesions of rabbits were studied to determine whether a similarity existed.

Five of the 14 strains reported in this series were tested in rabbits. The centrifugalized sediment of a 24 hour growth in 100 cc. broth was injected twice a week. In four rabbits injections were intravenous and in the fifth the streptococci were injected directly into the heart, effort being made to deliberately injure the valves to enable the organisms to obtain an early foothold there.

The work with these five strains isolated in cases of bacterial endocarditis agrees with what many investigators have shown, namely, that lesions grossly similar to human endocarditis can be produced in rabbits by injecting streptococci.

Two of the four rabbits injected intravenously over a period of four months showed vegetations on the mitral valves at necropsy. Two rabbits failed to show any evidence of pathological change in the heart valves or myocardium. The removal of the organisms before damage to the heart valves was accomplished, may probably be explained by the high antibody content of the serum of these rabbits. The rabbit which received three large injections directly into the heart at monthly intervals showed vegetations on the mitral and tricuspid valves.

The microscopic appearance of the valvular lesions was similar in all cases and also similar to the lesions studied in several human cases. The lesion seemed to begin within the valve with proliferative and exudative inflammation. Large irregular mononuclear cells with vesicular nuclei were common in the cellular exudate. Multinucleated cells of the same type were frequently found. Edema of the tissue in

the early lesions was pronounced. Necrosis usually occurred in the nodular lesion and extended outward and destroyed the endothelium. Thrombosis in a slight degree occurred in practically all cases. The lesions were essentially the same as the human endocarditis lesions.

TOXIN PRODUCTION

The search for a soluble toxin was pursued in the hope of finding a common poison among the members of a group of organisms isolated from similar pathological lesions, having similar morphological and cultural characteristics but differing in their serological reactions.

Each strain was grown four days in infusion broth at 37.5 C. The cultures were then filtered through a Mandler filter 4 and the filtrates after being tested for sterility were injected into mice. Two cc. of every filtrate failed to kill the animals.

Two strains (4 and 9) were then grown in infusion broth for 14 days and mice injected daily during this period to determine whether toxin might be formed in a longer time than that used in the preceding experiment. No fatalities resulted, however.

Skin tests were also performed on patients suffering from endocarditis. The filtrates of stock strains were injected intradermally in dilutions 1 : 1000, 1 : 500 and undiluted, with negative results. In seven cases the organism isolated from the particular individual tested was used in preparing the filtrates and the tests performed with the same dilutions as above. These also yielded negative results. Readings were taken 6 and 24 hours after injection. It appears from these results that no real exotoxin is secreted by these members of the viridans group associated with endocarditis.

It was thought that possibly an endotoxin or split protein product derived from the bacterial cell might be the factor giving rise to the pathological condition in endocarditis. Hence the attempt was made to liberate this apparent poisonous substance from the viridans cell by means of proteolytic enzymes. A 1% solution of trypsin in 0.85% NaCl was passed through a Mandler filter, 4. This filtrate was mixed with an equal volume of a heavy suspension of strain 11 in buffer solution (P_H 7.6), containing a 24 hour growth of 11 in 100 cc. broth which was centrifugated and resuspended in 5 cc. of the buffer solution. Mice were injected with 0.5 cc. and 1 cc. of this mixture daily for a period of ten days. All the mice lived. Guinea-pig serum and the filtrates of both cultures of proteolytic organisms as Serratia prodigiosa (Bacillus prodigiosus), Proteus vulgaris and other gram

negative bacilli capable of liquefying gelatin were used in place of trypsin. Only negative results were obtained. The same experiments were tried using 4 and 8, but all efforts to liberate an endotoxin or poisonous split protein were unavailing.

SUMMARY

In a relatively high percentage of cases of endocarditis a streptococcus can be isolated from the blood; and also frequently from vegetations on heart valves and infarcts in cases coming to necropsy.

In the cases studied, all but one yielded an organism producing green colonies on blood agar. In the one exception a typical beta hemolyzer was isolated.

None of the strains liquefied gelatin.

All strains produced acid in litmus milk.

The fermentation of carbohydrates was found to be irregular. No satisfactory classification can be made on this basis.

The streptococci are insoluble in bile and of low virulence.

Cultivation of organisms in collodion sacs in the peritoneal cavity of rabbits did not change their morphologic and cultural characteristics, or their serologic reactions.

There occurred no cross-agglutination, cross-precipitation or cross-complement fixation among the strains isolated. From these observations and the fact that no absorption of agglutinins took place, it appears impossible to group these streptococci on the basis of their serological reactions.

Immune bodies for the homologous strain were found in the blood of all patients tested.

Lesions of the heart valves similar to those occurring in human beings can be produced experimentally in animals by injection of these organisms directly into the heart and intravenously.

It appears that no exotoxin, endotoxin, or poisonous split protein can be liberated from the strains studied.

When the foregoing facts are considered, there seems to be ground for the belief that endocarditis with its associated lesions is produced by streptococci, usually members of the viridans group. This statement is based on the fact that inoculation of rabbits with cultures of the groups studied has led to the development of valvular lesions comparable to those seen in human beings.

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